CHAPTER 14

AFLACARD T20 TEST KIT

14.1 GENERAL INFORMATION

The AFLACARD T20 test kit is a qualitative enzyme immunoassay procedure for the detection of total aflatoxins. The test provides qualitative (less than or equal to a specified threshold) results.

14.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the AFLACARD T20 test method is a methanol/water (distilled or deionized) mixture consisting of 80 percent methanol (Reagent grade or better) and 20 percent water.

a. Using a graduated cylinder, measure 800 ml of methanol and place it into a clean carboy with spigot.

b. Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

c. Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

d. Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts of deionized or distilled water.

14.3 PREPARATION OF TESTING MATERIALS

a. Conjugate.

(1) Add 2 ml of conjugate diluent buffer (pink label) to the freeze-dried conjugate (amber vial).

(2) Replace the rubber cap and mix gently by inversion.

(3) Transfer all of the conjugate into the empty conjugate dropper bottle (red label) and write the preparation date on the label.

(4) **Leave the conjugate at room temperature for at least 30 minutes before use.**

NOTE: The ready to use conjugate is stable at 36° - 46° F

b. Other Kit Components.

(1) Remove the AFLACARD T20 kit from the refrigerator and leave the test kit components; substrate (blue label), wash buffer (green label), substrate (blue label), stop solution (yellow label), and test card at room temperature for at least 30 minutes before using the test.

Each card has two ports and therefore can perform two tests. The second port should be used within 8 hours of the first port. Each port has a sample area and control area. Please ensure the airholes on the card are on the right hand side and are not blocked or covered during the assay.

(2) Check that the two ports on the card to be used each exhibit two light blue spots.

NOTE: Each unused card has two light blue spots on each port which will disappear during the course of the assay.

14.4 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the (80/20) methanol/water extraction solvent.

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Remove the cover and funnel a minimum of 10 ml of the extract through a Whatman No.4 filter paper into a sample jar labeled with the sample identification.

e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container.

14.5 TEST PROCEDURES

a. Sample Preparation.

(1) Remove the lid from the sample diluent tube and add 200 µl of filtrate.

(2) Cap and invert sample diluent tube. The sample is now ready to be applied to the card.

b. Sample Analysis.

(1) Apply 250 µl of diluted filtrate to sample port and incubate for a minimum of 1 minute. Always ensure that the liquid has passed completely through the membrane before proceeding to the next step.

(2) Using the conjugate dropper bottle (red label), apply 3 drops of conjugate to the test port and incubate for 1 minute.

(3) Using the wash buffer dropper bottle (green label), apply 3 drops of wash buffer to the test port and incubate for 1 minute.

(4) Dry around the port with a tissue.

(5) Using the substrate dropper bottle (blue label), apply 3 drops of substrate to the test port and incubate for 2 minutes.

(6) Using the stop solution dropper bottle (yellow label), apply 3 drops of stop solution to the test port. Allow the solution to pass completely through the membrane.

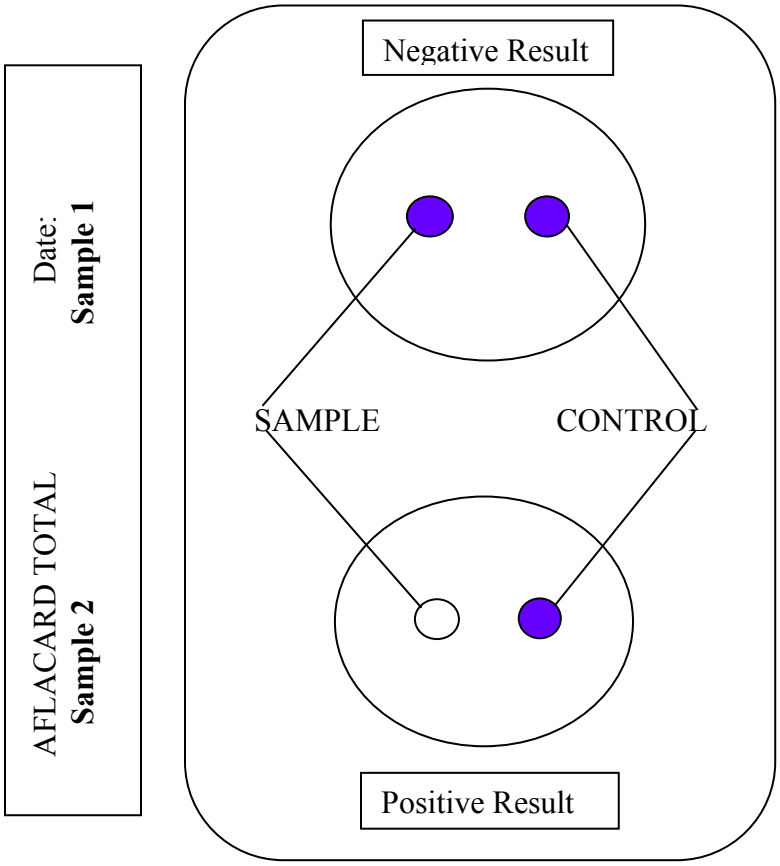
c. Reading Test Results.

(1) The control spot must develop a **clearly visible purple color** in order to have a valid test result. The color in the sample and the control spot does not need to be of the same intensity.

(2) The sample should be considered to be negative when the sample and control spot both have clearly visible color development.

(3) The sample should be considered to be positive (more than 20 ppb) when there is no detectable color on the sample spot.

INTERPRETATION OF RESULTS



14.6 REPORTING AND CERTIFYING RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than a threshold (e.g., 20 ppb) or as exceeding the threshold.

b. Certify results as being equal to or less than a threshold.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

14.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

14.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

14.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits:

(1) 10 Aflacard Total Cards.

(2) 20 tubes containing 3.8 ml Sample Diluent Buffer.

(3) 2 Freeze-dried Conjugate vials (red label).

(4) 1 Conjugate Diluent Buffer vial (pink label).

(5) 2 Conjugate Dropper Bottles (empty, red label).

(6) 1 Wash Buffer dropper bottle (green label).

(7) 1 Substrate dropper bottle (blue label).

(8) 1 Stop Solution dropper bottle (yellow label).

b. Materials Required but not Provided:

(1) Sample grinder.

(2) Balance.

(3) Methanol - ACS grade.

(4) Distilled or deionized water.

(5) Blender with mixing jars.

(6) Timer.

(7) Whatman No.4 Filter Paper.

(8) Tissue paper.

(9) Sample collection container.

14.10 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 36° - 46° F. **Do not freeze.**

b. Precautions.

(1) Do not use kit components beyond the expiration date.

(2) Do not use reagents from one batch number in conjunction with reagents from a different batch number, and do not substitute reagents from other manufacturers.

(3) Kits should be brought to room temperature (68° - 82° F) prior to use. This will take approximately 30 minutes.